

Amendments T The Claims

The following listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS

1. (currently amended) A method for improving a genetic stability of a foreign insert nucleotide sequence in a recombinant ~~single-stranded RNA-virus~~ poliovirus vector, which comprises performing a mutagenesis of the foreign insert nucleotide sequence (a) to provide even distribution of G/C content throughout the overall foreign insert nucleotide sequence and/or (b) to increase G/C content of the foreign insert without substantially causing amino acid substitutions.

2-3. (cancelled).

4. (original) The method according to claim 1, wherein the mutagenesis renders the foreign insert nucleotide sequence to have the G/C content of more than 30%

5. (original) The method according to claim 4, wherein the mutagenesis renders the foreign insert nucleotide sequence to have the G/C content of more than 40%.

6. (original) The method according to claim 1, wherein the mutagenesis of the insert nucleotide sequence to provide even distribution of G/C content is performed by increasing G/C content of local A/T-rich region in the foreign insert nucleotide sequence.

7. (original) The method according to claim 6, wherein the mutagenesis renders the local A/T-rich region of the foreign insert nucleotide sequence to have the G/C content of more than 30%.

8. (original) The method according to claim 7, wherein the mutagenesis renders the local A/T-rich region of the foreign insert nucleotide sequence to have the G/C content of more than 40%.

9. (currently amended) The method according to any one of claims ~~1-8~~ 1, 4, 5, 6, 7 and 8, wherein the mutagenesis is performed by silent mutations.

10. (currently amended) The method according to any one of claims ~~1-8~~ 1, 4, 5, 6, 7 and 8, wherein the foreign insert nucleotide sequence is smaller than 480 bp in size.

11. (currently amended) The method according to claim 10, wherein the foreign insert nucleotide sequence is smaller than ~~480~~ 450 bp in size.

12. (currently amended) The method according to claim ~~3~~ 1, wherein the poliovirus is one selected from the group consisting of poliovirus type 1, poliovirus type 2 and poliovirus type 3.

13. (currently amended) The method according to claim ~~3~~ 1, wherein the poliovirus is one selected from the group consisting of poliovirus Sabin type 1, poliovirus Sabin type 2 and poliovirus Sabin type 3.

14. (original) The method according to claim 13, wherein the poliovirus is poliovirus Sabin type 1.

15. (original) The method according to claim 1, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen selected from the group consisting of bacterial polypeptide antigens, viral polypeptide antigens, fungal polypeptide antigens and eukaryotic parasite polypeptide antigens.

16. (original) The method according to claim 15, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen of an infectious virus selected from human immunodeficiency virus, simian immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, human papilloma virus, herpes simplex virus, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

17. (original) The method according to claim 16, wherein the polypeptide or a protein antigen is derived from the coding region covering the antigenic determinant sites.

18. (currently amended) The method according to claim 15 or 16, wherein the foreign insert nucleotide sequence encoding the polypeptide antigen is ~~monomeric~~, dimeric or multimeric.

19. (currently amended) The method according to claim 18, wherein the dimeric or multimeric foreign insert is homo/~~hetero-dimer~~hetero-dimer or homo/hetero-multimer.

20. (currently amended) A method for constructing ~~a recombinant single stranded RNA virus~~ a recombinant poliovirus containing ~~an a foreign~~ insert nucleotide sequence, which comprises the steps of:

(a) ~~preparing the foreign insert nucleotide sequence which has an even distribution of G/C content which has an even distribution of G/C content~~

~~throughout the overall foreign insert nucleotide sequence and/or has a G/C content of more than 30%; and~~

(a) performing a mutagenesis of the foreign insert nucleotide sequence (i) to provide even distribution of G/C content throughout the overall foreign insert nucleotide sequence and/or (ii) to increase G/C content of the foreign insert without substantially causing amino acid substitutions; and

(b) introducing the foreign insert into a viral genome of a parent RNA virus to construct the recombinant ~~RNA virus poliovirus~~,

wherein the foreign insert nucleotide sequence is introduced in such a manner that the ~~recombinant RNA virus recombinant poliovirus~~ is not disrupted for viral propagation

21. (withdrawn)

22-24. (cancelled)

25. (original) The method according to claim 20, wherein the foreign insert nucleotide sequence has the G/C content of more than 40%.

26. (currently amended) The method according to claim ~~22-20~~, wherein the mutagenesis of the foreign insert nucleotide sequence to provide even distribution of G/C content is performed by increasing G/C content of local A/T-rich region of the foreign insert nucleotide sequence.

27. (original) The method according to claim 26, wherein the mutagenesis at a local A/T-rich region renders the region to have the G/C content of more than 30%.

28. (original) The method according to claim 27, wherein the mutagenesis at a local A/T-rich region renders the region to have the G/C content of more than 40%.

29. (currently amended) The method according to claim ~~22-20~~, wherein the mutagenesis is performed by silent mutations.

30. (currently amended) The method according to any one of claims ~~20-29~~, 25, 26, 27, 28 and 29, wherein the insert nucleotide sequence is smaller than 480 bp in size.

31. (original) The method according to claim 30, wherein the foreign insert nucleotide sequence is smaller than 450 bp in size.

32. (currently amended) The method according to claim ~~23-20~~, wherein the poliovirus is one selected from the group consisting of poliovirus type 1, poliovirus type 2 and poliovirus type 3.

33. (currently amended) The method according to claim ~~23-20~~, wherein the poliovirus is one selected from the group consisting of poliovirus Sabin type 1, poliovirus Sabin type 2 and poliovirus Sabin type 3.

34. (original) The method according to claim 33, wherein the poliovirus is poliovirus Sabin type 1.

35. (original) The method according to claim 20, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen selected from the group consisting of bacterial polypeptide antigens, viral polypeptide antigens, fungal polypeptide antigens and eukaryotic parasite polypeptide antigens.

36. (original) The method according to claim 35, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen of an infectious virus selected from human immunodeficiency virus, simian immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, human papilloma virus, herpes simplex virus, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

37. (original) The method according to claim 36, wherein the polypeptide or a protein antigen is derived from the coding region covering the major or minor antigenic determinant sites.

38. (currently amended) The method according to claims 36 or 37, wherein the foreign insert nucleotide sequence encoding the polypeptide antigen is ~~monomeric~~, dimeric or multimeric.

39. (currently amended) The method according to claim 38, wherein the dimeric or multimeric foreign insert is homo/~~hetero-dimer~~-hetero-dimer or homo/hetero-multimer.

40. (currently amended) A recombinant ~~single stranded RNA virus poliovirus~~ comprising ~~an a~~ foreign insert nucleotide sequence, characterized in that the recombinant ~~single stranded RNA virus poliovirus~~ is constructed by the method according to any one of claims 20-29, 25, 26, 27, 28 and 29.

41. (currently amended) The recombinant ~~single stranded RNA virus poliovirus~~ according to claim 40, wherein the foreign insert nucleotide sequence is smaller than 480 bp in size.

42. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 41, wherein the foreign insert nucleotide sequence is smaller than 450 bp in size.

43. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 40, wherein the poliovirus is one selected from the group consisting of poliovirus type 1, poliovirus type 2 and poliovirus type 3.

44. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 43, wherein the poliovirus is one selected from the group consisting of poliovirus Sabin type 1, poliovirus Sabin type 2 and poliovirus Sabin type 3.

45. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 44, wherein the poliovirus is poliovirus Sabin type 1.

46. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 40, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen selected from the group consisting of bacterial polypeptide antigens, viral polypeptide antigens, fungal polypeptide antigens and eukaryotic parasite polypeptide antigens.

47. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 46, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen of an infectious virus selected from human immunodeficiency virus, simian immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, human

papilloma virus, herpes simplex virus, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

48. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 47, wherein the polypeptide or the protein antigen is derived from the coding region covering the major or minor antigenic determinant sites.

49. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claims 47 or 48, wherein the foreign insert nucleotide sequence encoding the polypeptide antigen is ~~monomeric~~, dimeric or multimeric.

50. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to claim 49, wherein the dimeric or multimeric foreign insert is homo/hetero-dimer ~~dimmer~~ or homo/hetero-multimer.

51. (currently amended) The recombinant ~~single-stranded RNA virus~~ poliovirus according to ~~any one of claims 43-45~~ claim 40, wherein the recombinant poliovirus comprises:

- (a) a genomic nucleotide sequence of a parent poliovirus;
- (b) an additional polioviral cleavage site; and
- (c) the foreign insert nucleotide sequence,

wherein the foreign insert nucleotide sequence is introduced into the viral genome of a parent poliovirus without disrupting the viral infection and proliferation, and a poliovirus protease also acts on the additional cleavage site so that the polypeptide or protein antigen

encoded by the foreign insert nucleotide sequence is released from a polypeptide precursor of the recombinant poliovirus.

52-55. (withdrawn).